IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Henry M. Krause, et al.	§	Confirmation No. 5757
Serial No.:	10/531,095	§	Group Art Unit: 1635
Filed:	April 7, 2005	§	Examiner: Dana H. Shin
For:	Trap-Tagging: A Novel Method For the Identification and Purification of RNA-Protein Complexes	§	Atty. Dkt. No. 1889-00900

DECLARATION UNDER 37 CFR § 1.132
OF HENRY M. KRAUSE

PURPOSE OF DECLARATION

This declaration is made as evidence of the patentability of the above identified patent application and specifically for the purpose of overcoming a ground of rejection or requirement made in the Office Action dated November 13, 2007. This Declaration is intended to be submitted in support of a Response to that Office Action.

STATEMENT OF FACTS AND OPINIONS

I, Henry M. Krause, state as follows:

1. I am over 18 years of age and competent to make this Declaration.

2. I hold a Ph.D. in Biochemistry from the University of Alabama at Birmingham. I received my postdoctoral training in Switzerland at the University of Basel, and joined the University of Toronto (Toronto, Ontario, Canada) as an Assistant Professor in 1988. I was promoted to Full Professor in 2000, and became a Canadian Institutes of Health Research Senior Investigator in 2002. I have published approximately 40 maniscripts in peer reviewed journals such as *Cell, Nature, Genes and Development, EMBO J,* and *Development.*

3. In addition to teaching and scientific research, my professional activities also include supervising the laboratory research studies of a number of undergraduate and graduate level

students, postdoctoral fellows and research associates, ranging from aproximately 15-20 lab members. I have served on many grant panels and review boards, and am the current Canadian representative to the Society for Developmental Biology and the past Canadian representative to the Genetics Society of America. I am also the founder of InDanio Bioscience, a company that is using a live zebrafish screening system to identify new drugs that target the nuclear hormone receptor family of transcription transcriptions.

4. I am an inventor named in the above-identified patent application. I have read the specification and claims of the above-identified patent application and am familiar with the invention described therein. I have also read the references cited against this application and understand their teachings.

5. To achieve the functional isolation (the framework wherein the "tags" and "targets" are not being interfered with), the insulator sequences need to be such that relatively small loops or stems are formed by the identical nucleotides and flanking restriction sites. One skilled in the art would understand that A and U nucleotides of RNA do not hybridize as tightly as C and G nucleotides, hence the preference for A or U, vs. C or G as they will be less likely to interact strongly with the "tag" and "target" sequences, thus allowing for proper folding of the RNA fusion molecule components. Excessively long stretches of identical nucleotides would render the RNA fusion molecules highly unstable, difficult to express and largely inactive.

6. *Srisiwat et al.* were never successful at making a construct containing two functional tags. A construct containing two tags was used in their paper, but only one of the tags was functional. While the problem was obvious, the solution was not. Simply adding short spacers did not work for them and, in Applicants' experience, does not work in general. Short RNA spacers have complete freedom to bend, allowing the tags or bait elements to interact just as easily as if the spacers were absent. Furthermore, there is also significant risk that the introduced spacers can interact/hybridize with the tag or bait sequences, rendering them inactive. Applicants circumvented this major problem by designing spacers/insulators with physical properties that reduce their flexibility and render them non-interactive. Using a combination of palindromic restriction sites and short stretches of adenosines achieves this. The resulting insulators are sufficiently bulky and inflexible to functionally isolate each of the tags and the bait. In overcoming these challenges and producing functional, dual tag constructs, Applicants discovered that there are

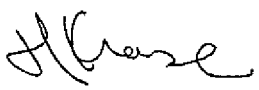
many reasons for failure, and very few solutions. To date, there are still no other successful RNA multi-tagging systems available.

7. Applicants were motivated to develop a multi-tagging system for RNP purification stemmed from their own work in protein purification. They found that using a combination of two or more different, reversible tags allowed them to achieve the purification levels required to isolate non-abundant proteins and associated cofactors from complex cells or tissues. They reasoned that it should be possible to develop an RNA equivalent for identifying RNA associated factors. The advantage of having two or more different tags in one construct, each capable of binding its unique matrix-bound ligand reversibly, was never previously recognized or described. Using different tags allows removal of factors that bind non-specifically to the each of the two matrices or tags. The usefulness of the system and the amount of information that can be gleaned is proportional to the strength and specificity of the tags used. At the time of development, the S1 tag was the only one available with affinity and selectivity values high enough to be useful. Other tags tested wherein the elements were merely separated by stretches of RNA resulted in low recovery levels of the target complex. To achieve the required purification level, significant modification of the second RNA tag and addition of the insulator elements was necessary to produce a fusion molecule according to the present application.

ACKNOWLEDGEMENT

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: March 10, 2008



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